

# Myxobacteria vs. Sponge-Derived Alkaloids: The Bengamide Family Identified as Potent Immune Modulating Agents by Scrutiny of LC-MS/ELSD Libraries

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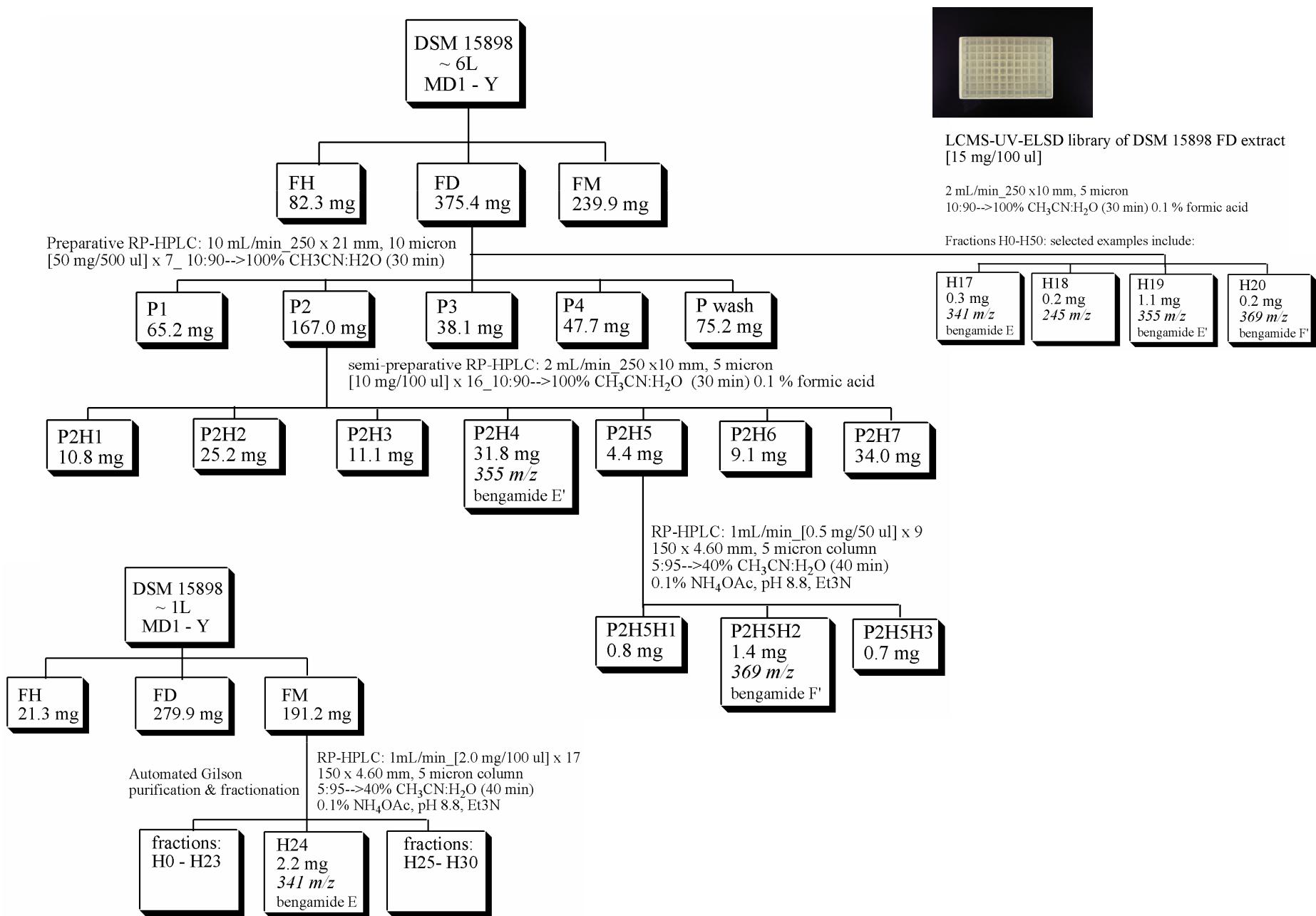
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## [Supporting Information] R6

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**Scheme S1.** Isolation schemes with LCMS-UV-ELSD library active fractions bengamides E (**8**), E' (**13**) and F' (**14**) from *M. virescens* strain DSM 15898.



**Table S1.**  $^1\text{H}$ ,  $^{13}\text{C}$ , COSY, HMBC and 1D NOESY NMR<sup>a</sup> Data of Bengamide E' diastereomers (a (60%), b (40%)) in  $\text{CDCl}_3$  and select  $^1\text{H}$  data in benzene  $d_6$

No.	Type <sup>b</sup>	mult	$\delta_H$ ( $\text{CDCl}_3$ )	$J$ , Hz ( $\text{CDCl}_3$ )	$\delta_H$ (benz. $d_6$ )	$J$ , Hz (benz. $d_6$ )	$\delta_c$ ( $\text{CDCl}_3$ )	gHMBC	gCOSY	1D NOESY
1	$\text{CH}_2$	m	1.31				29.6	3	2,15,17	
2	CH	m	2.02				38.3			
3a	CH	ddd	5.68	1.2, 7.2, 15	5.79	1.2, 7.8, 15.6	140.8	1, 2, 5	2, 4	
3b		ddd	5.70	1.2, 7.2, 15	5.80	1.2, 7.8, 15.6				
4a	CH	ddd	5.45	1.2, 7.2, 15	5.69	1.2, 6.6, 15.6	126.9	2, 5, 6	3, 5	
4b		ddd	5.46	1.2, 7.2, 15	5.70	1.2, 6.6, 15.6				
5	CH	dd	4.22	6.6, 6.0			74.5	3, 4, 6, 7	4,6	
6	CH	ddd	3.60	1.2, 1.2, 5.4			72.9	4, 5	5	
7	CH	ddd	3.84	1.8, 1.8, 7.2			72.8		6	
8	CH	dd	3.79	6.0, 6.6			81.4	6, 7		
9	C						172.2			
10	CH	ddd	4.53	1.2, 7.2, 10.8			52.2	9, 11, 16	11', NH	11, 11' 12, 14
11	$\text{CH}_2$	m	2.02				31.3			
11'			1.57					10, 12, 16	10, 11 12', 13'	
12	$\text{CH}_2$	m	2.02				28.1			
12'		m	1.80						14	
13	$\text{CH}_2$		1.80				29.0			
13'			1.42						12, 14	
14	$\text{CH}_2$	m	3.28				42.3		12, 12', 13	10, 11, 12
15a	$\text{CH}_3$	d	0.99	6.6	0.95	6.6	20.0		1, 2, 17	1, 2, 3, 4, 17
15b		d	0.98	6.6	0.94	6.6				
16	C						175.1			
17a	$\text{CH}_3$	t	0.85	7.8	0.85	7.8	12.0		1, 2, 15	
17b		t	0.86	7.8	0.86	7.2				
	$\text{OCH}_3$	s	3.52				60.0			

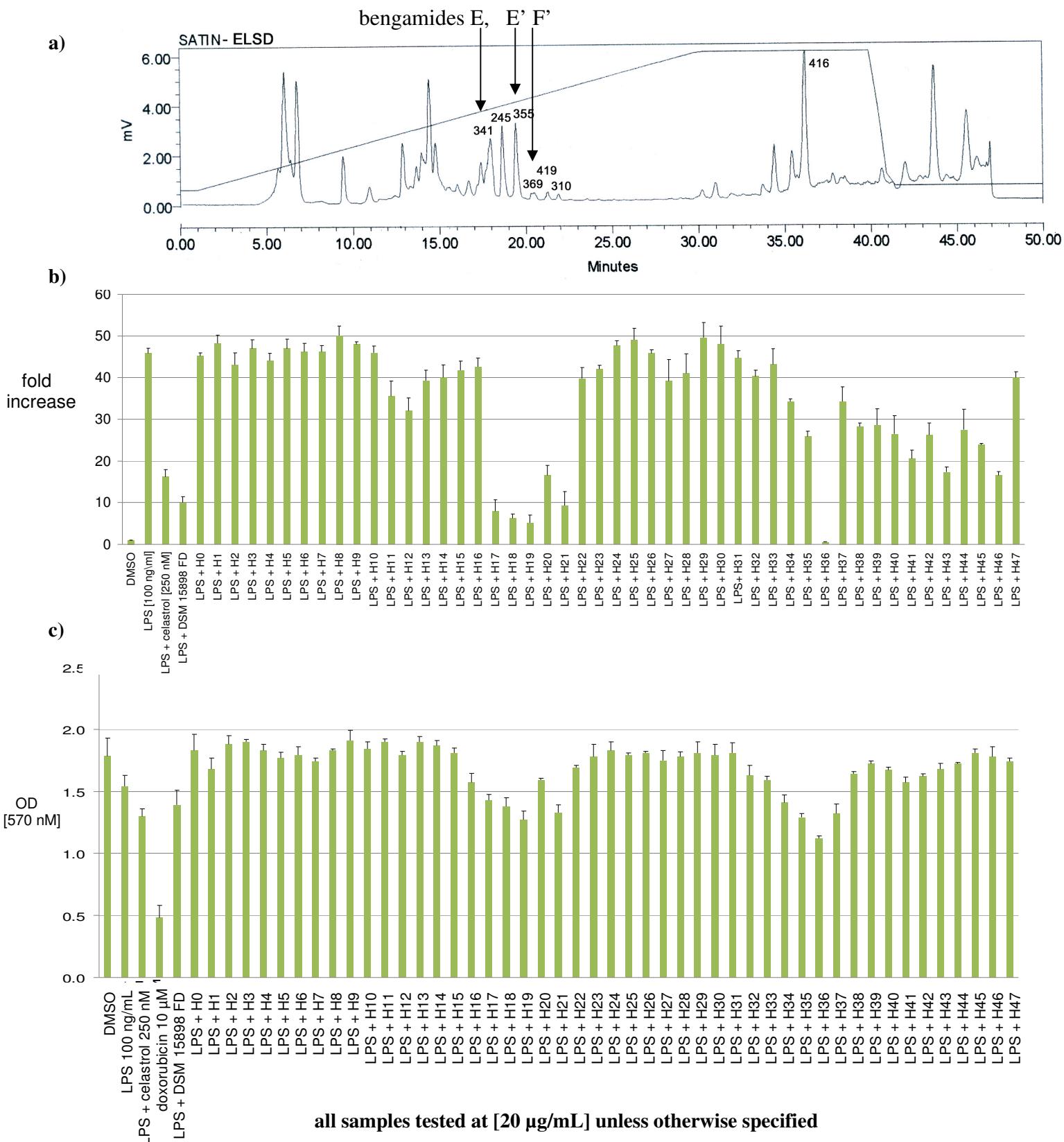
<sup>a</sup>Measured at 600 MHz ( $^1\text{H}$ ) and 125 MHz ( $^{13}\text{C}$ ). <sup>b</sup>Carbon type determined by DEPT and HMQC experiments.

**Table S2.**  $^1\text{H}$ , and  $^{13}\text{C}$  NMR Data <sup>a</sup> of bengamide F' (**14**) in  $\text{CDCl}_3$ .

No.	Type <sup>b</sup>	$\delta_H$	mult.	$J$ (Hz)	$\delta_c$
1	$\text{CH}_2$	1.31	m		29.4
2	CH	2.02	m		38.1
3	CH	5.68	dd	15.6, 7.8	140.4
4	CH	5.45	dd	15.6, 7.2	126.6
5	CH	4.23	t	6.0	74.3
6	CH	3.61	m		72.3
7	CH	3.82	appdd	6.0, 1.2,	72.3
8	CH	3.78	d	7.2	80.8
9	C				172.1
10	CH	4.62	dd	11.4, 6.6	55.0
11	$\text{CH}_2$	2.02	m		31.2
11'		1.51	m		
12	$\text{CH}_2$	2.02			27.7
12'		1.82			
13	$\text{CH}_2$	1.82			26.6
13'		1.44			
14	$\text{CH}_2$	3.21	dd	15.0, 5.4	52.4
15	$\text{CH}_3$	0.98	d(2)	6.6	19.8
16	C				171.9
17	$\text{CH}_3$	0.85	t(2)	7.8	11.8
	$\text{OCH}_3$	3.55	s		60.0
	$\text{NCH}_3$	3.05	s		36.1
	N-H	8.12	s		

<sup>a</sup>Measured at 600 MHz ( $^1\text{H}$ ) and 125 MHz ( $^{13}\text{C}$ ).

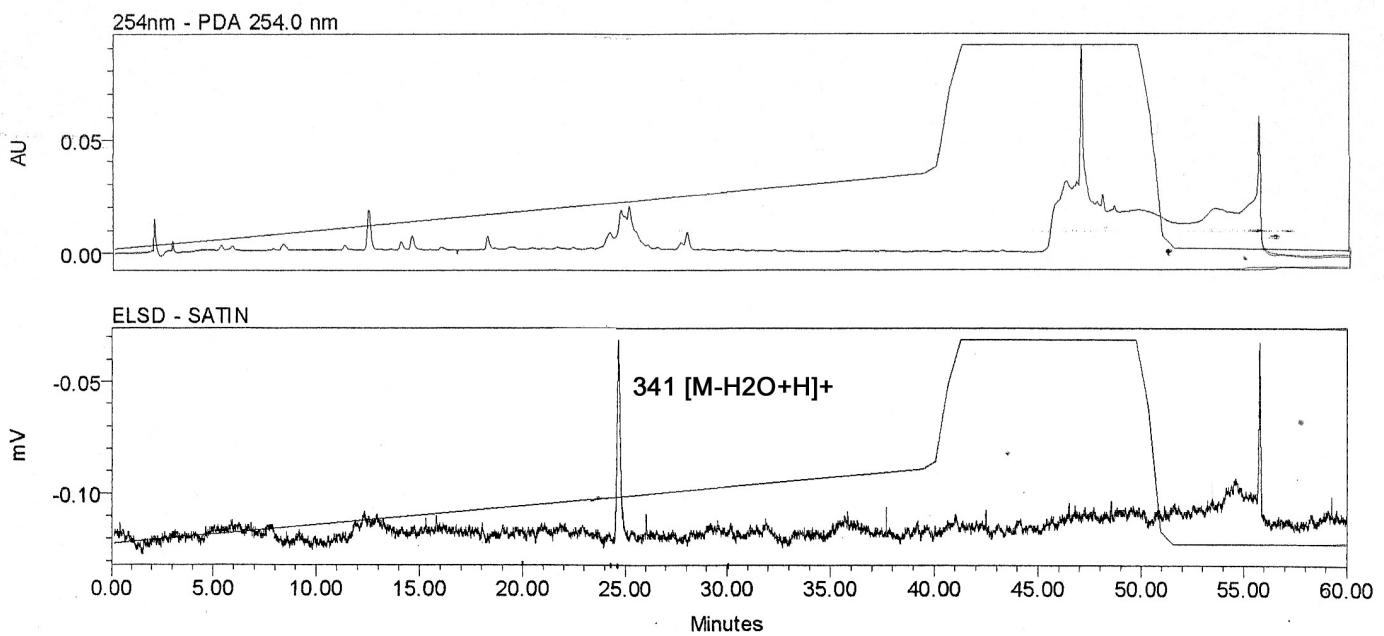
appdd = apparent dd



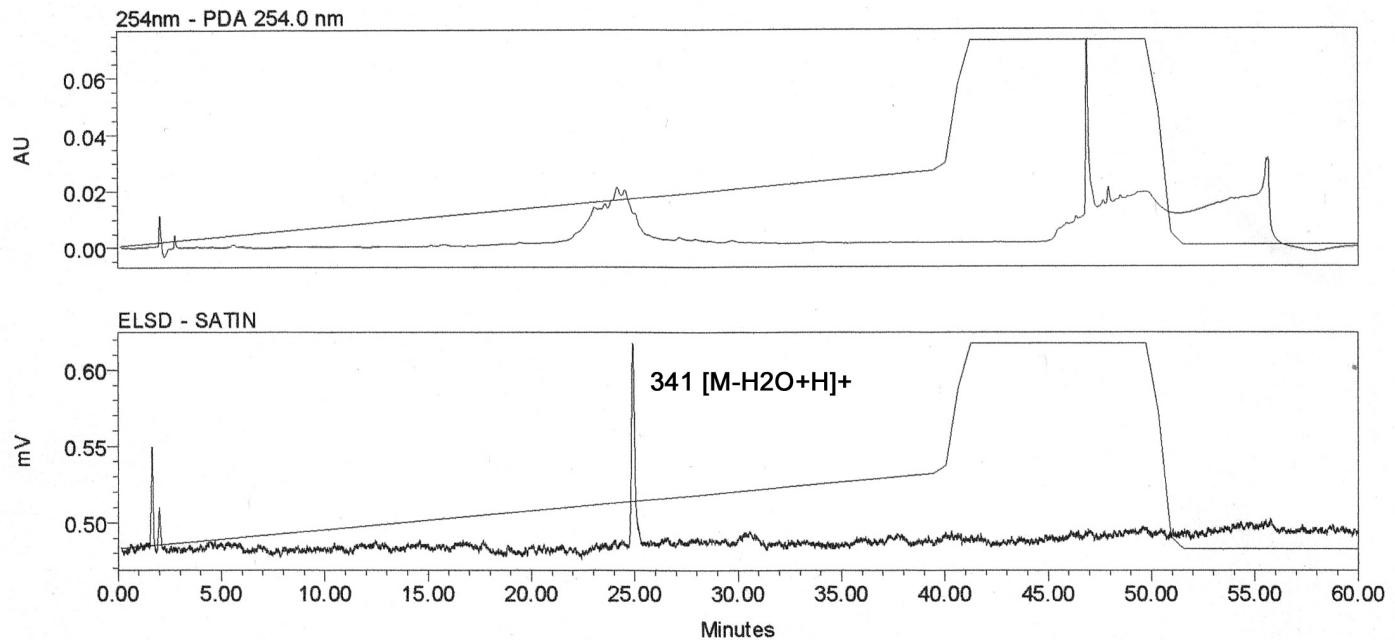
**Figure S1.** Observed LCMS-ELSD library trace with annotations including selected  $m/z$  ions (a), NFkB luciferase assay activity (b), and cytotoxicity data against murine macrophage (RAW 264.7) cells (c) from *M. virescens* sample extract coded DSM 15898 FD. Assays (b) and (c) underwent 18h incubation times.

a)

## Overlaid Chromatogram

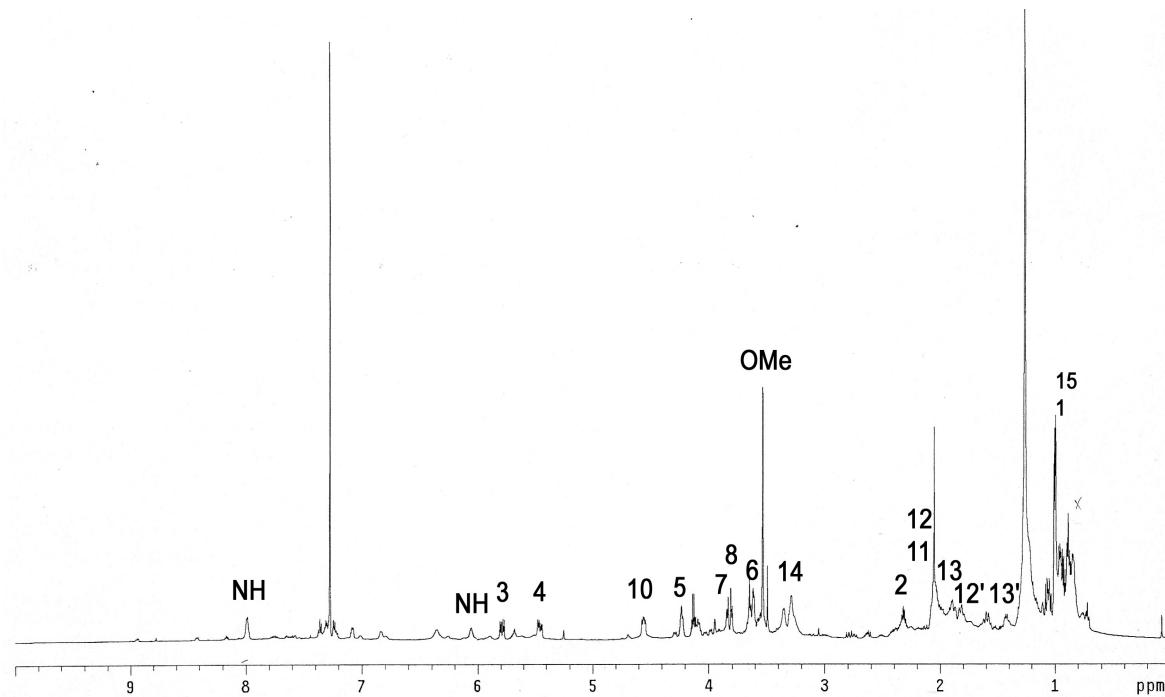


b)

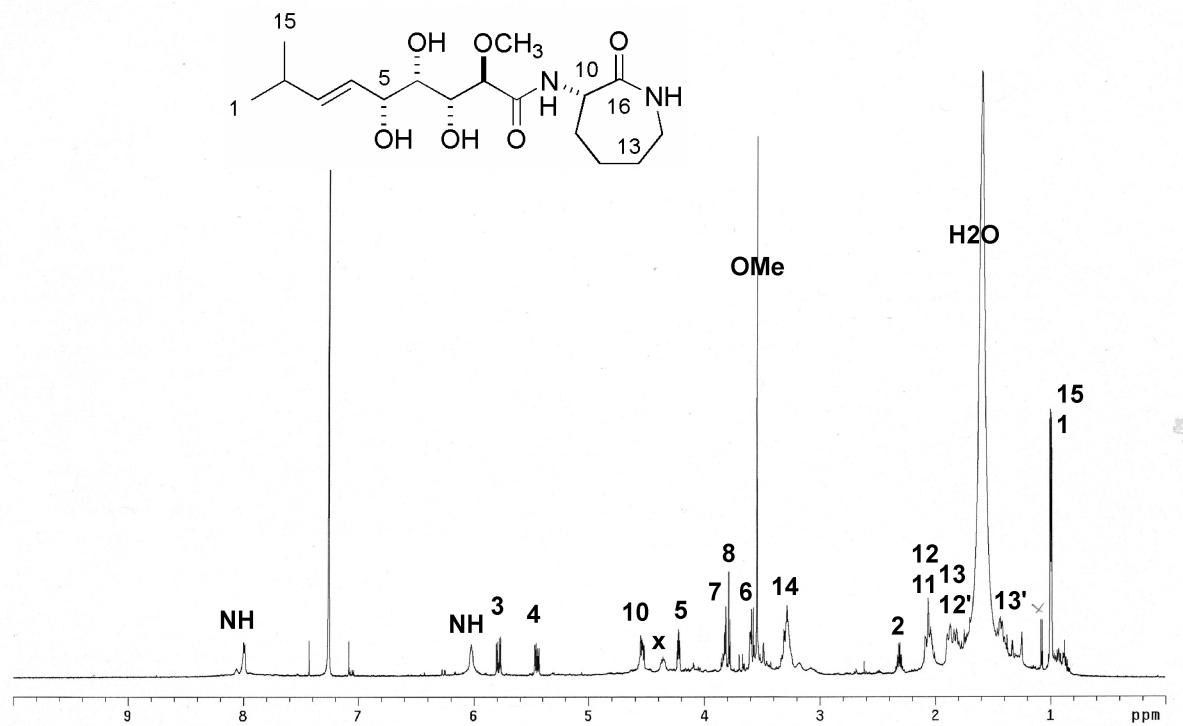


**Figure S2.** Comparative LCMS-UV-ELSD traces of: (a) fraction DSM 15898 FM H24 isolated from *M. virescens* versus (b) bengamide E (**8**) isolated from *J. coriacea* with annotations including m/z ions.

a)

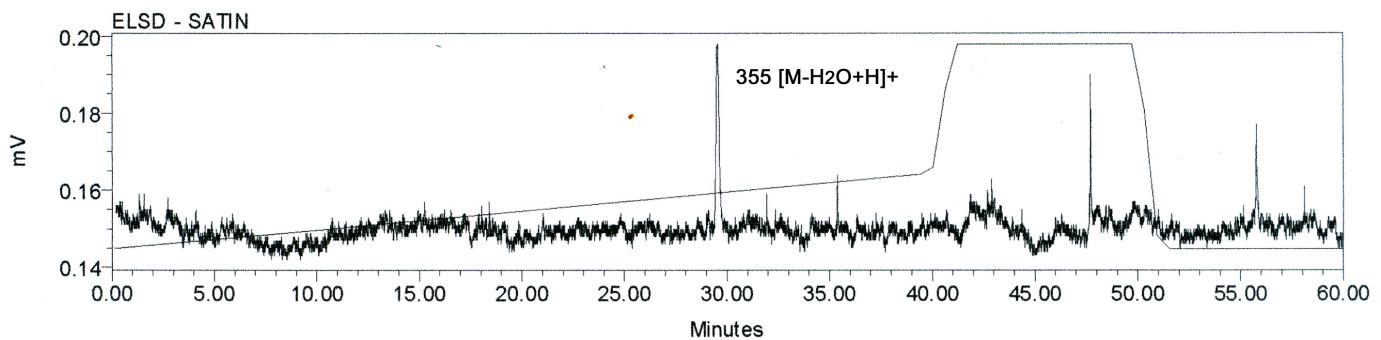


b)

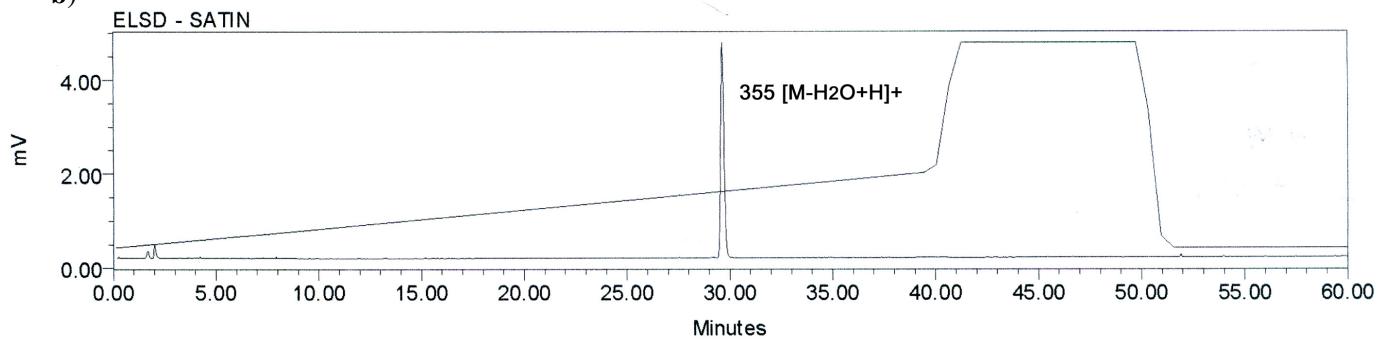


**Figure S3.** Comparative <sup>1</sup>H NMR spectrum of: (a) scale up fraction of DSM 15898 FM H24 isolated from *M. virescens* versus (b) bengamide E (**8**) isolated from *J. coriacea* (600 MHz, CDCl<sub>3</sub>).

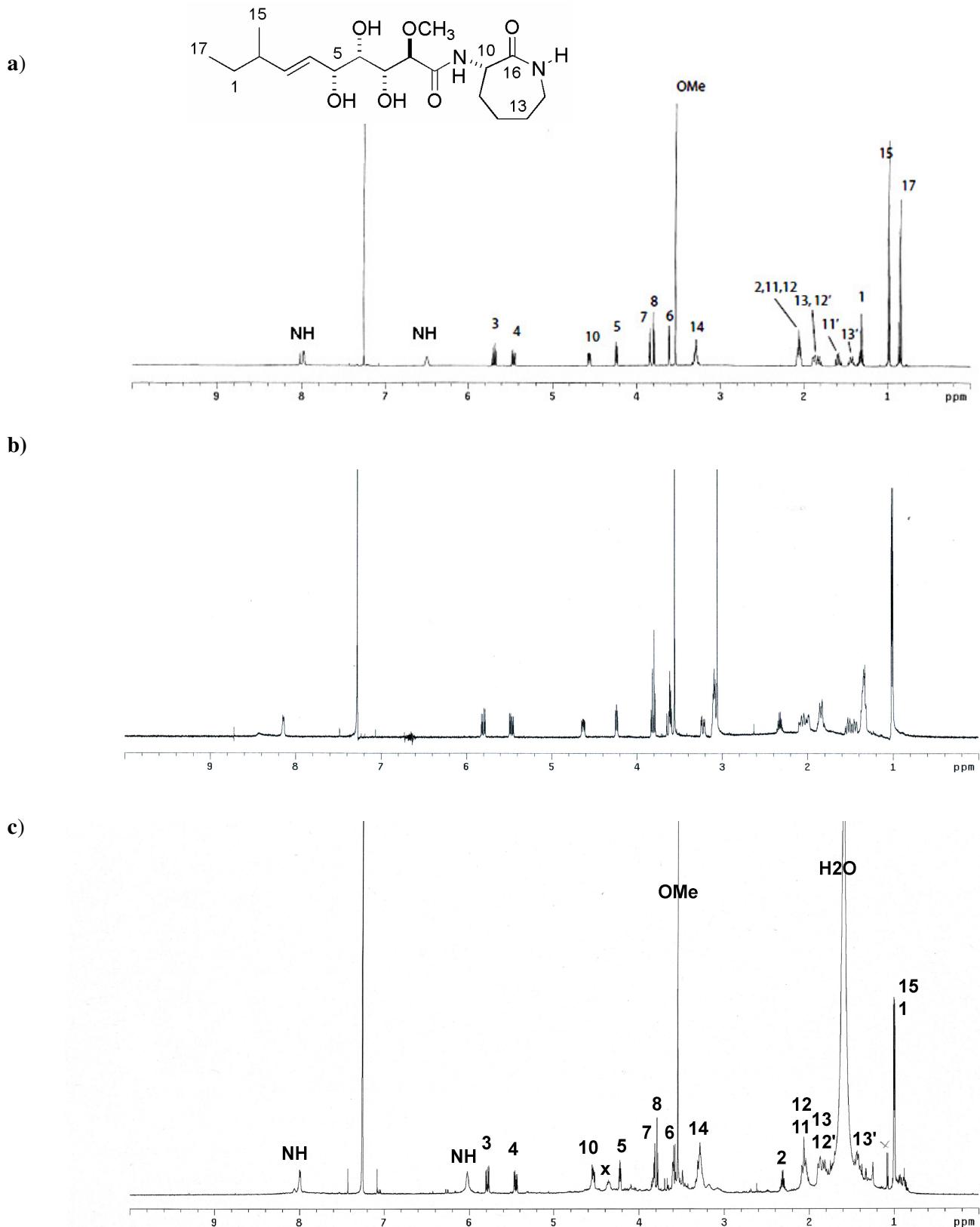
a)



b)

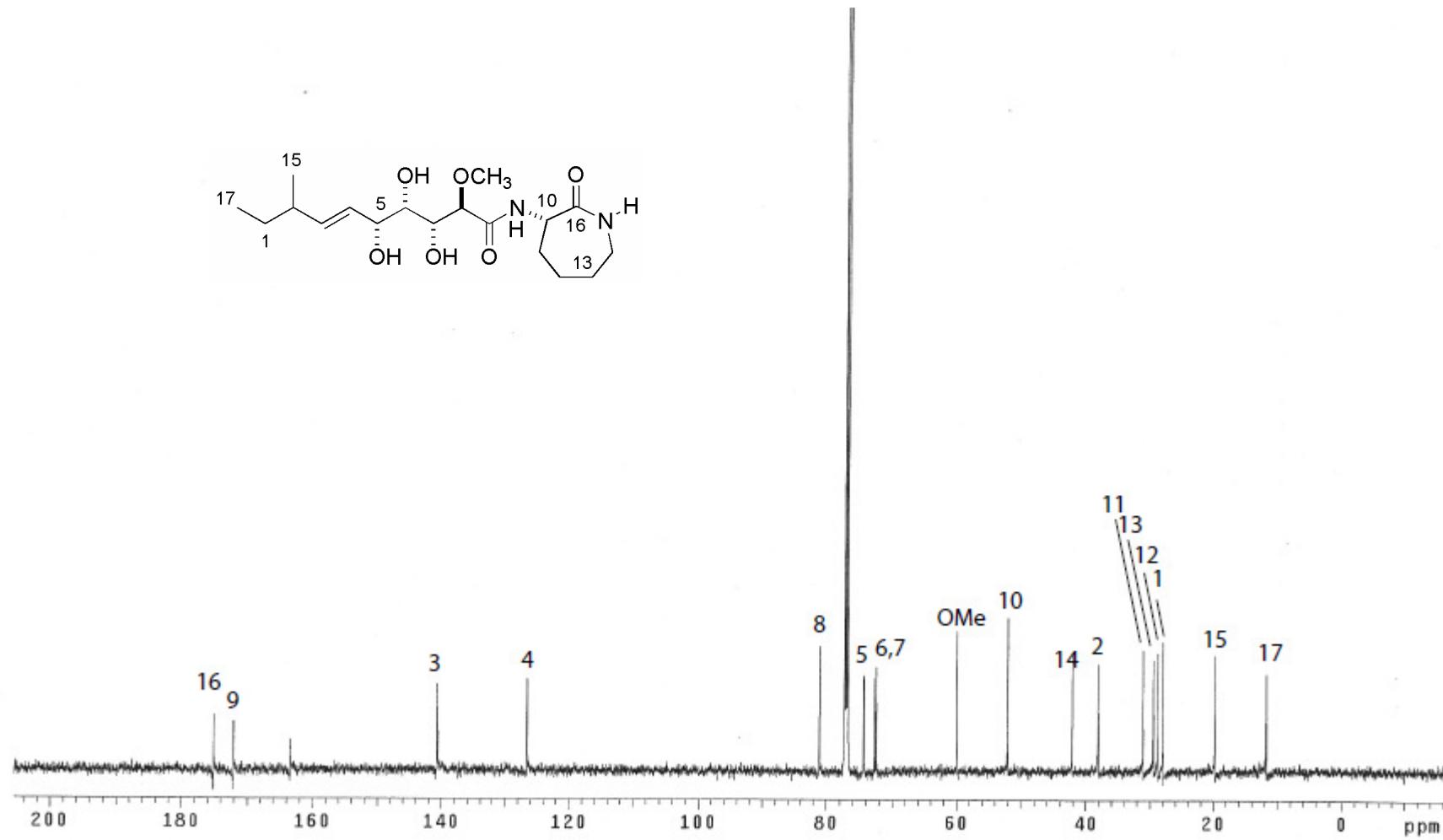


**Figure S4.** Comparative of LCMS-ELSD traces of: (a) library fraction DSM 15898 FD H19 bengamide E' (**13**) versus (b) bengamide F (**14**) isolated from *J. coriacea* with annotations including m/z ions.

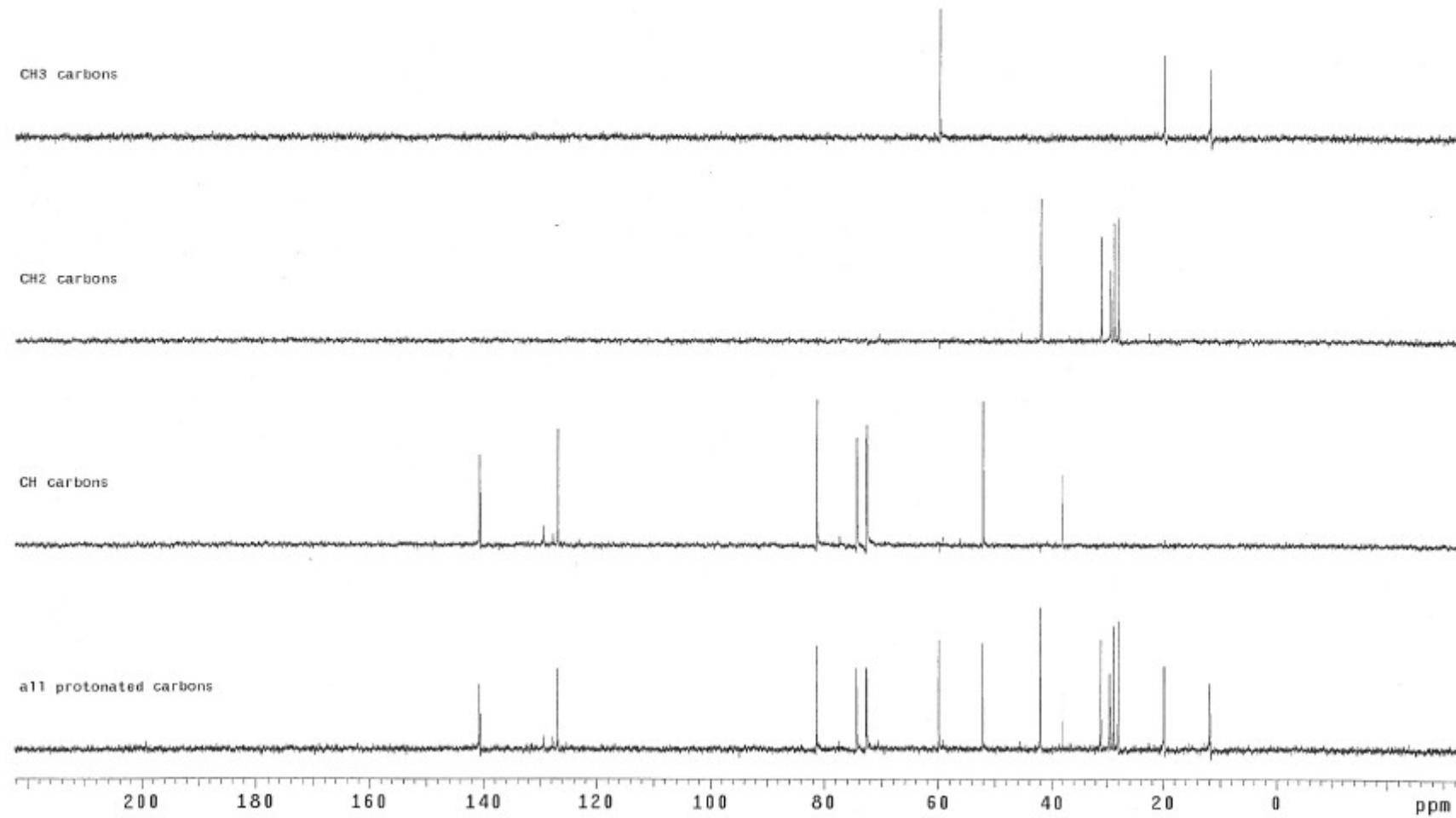


**Figure S5.** Comparative  $^1\text{H}$  NMR spectrum of: (a) library fraction DSM 15898 FD H19 (bengamide E', **13**) isolated from DSM 15898 versus (b) bengamide F (**9**) and (c) bengamide E (**8**) isolated from *J. coriacea* (600 MHz,  $\text{CDCl}_3$ ).

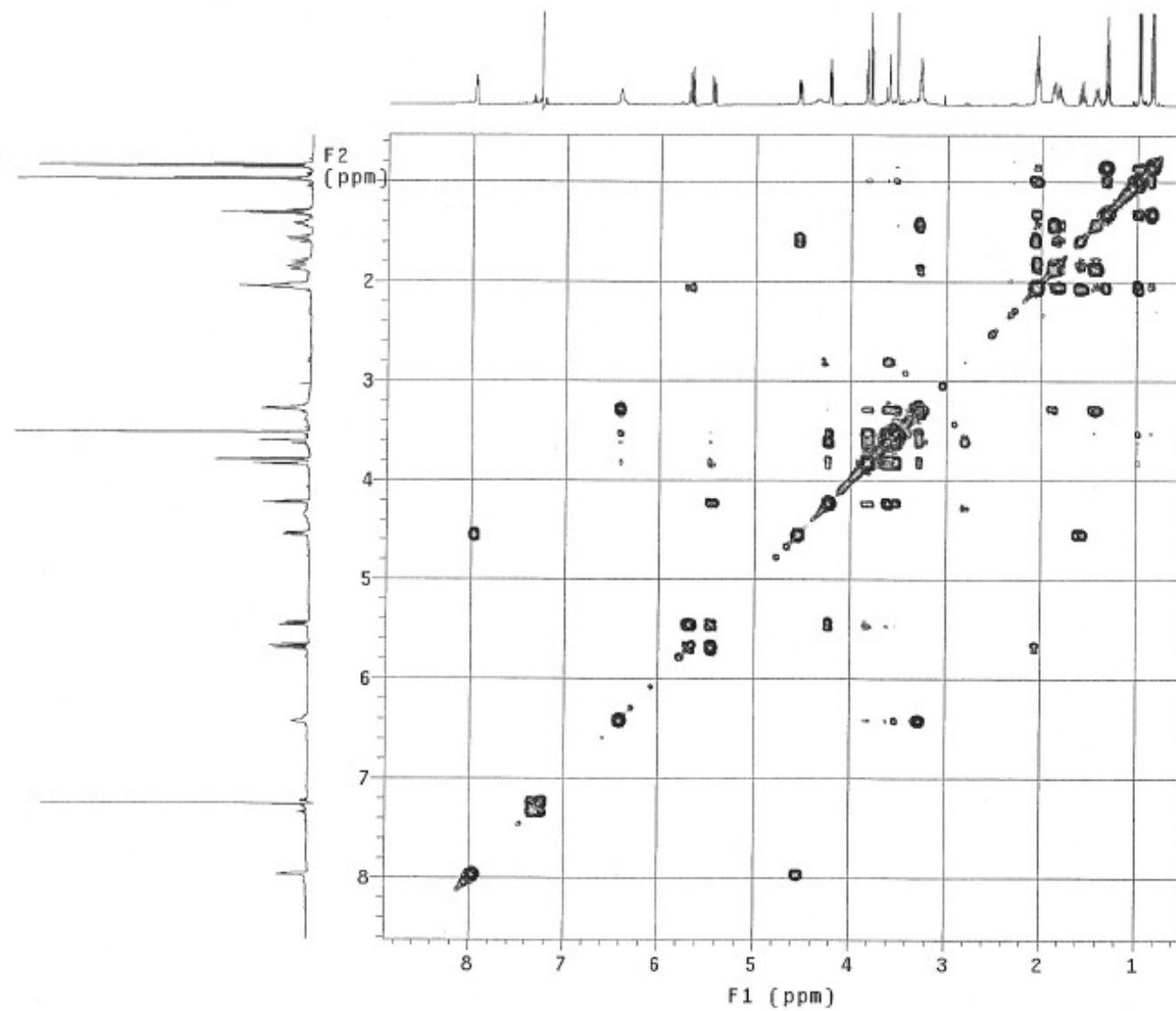
**Figure S6.**  $^{13}\text{C}$  NMR spectrum of bengamide E' (**13**), (600 MHz,  $\text{CDCl}_3$ ) isolated from DSM 15898.



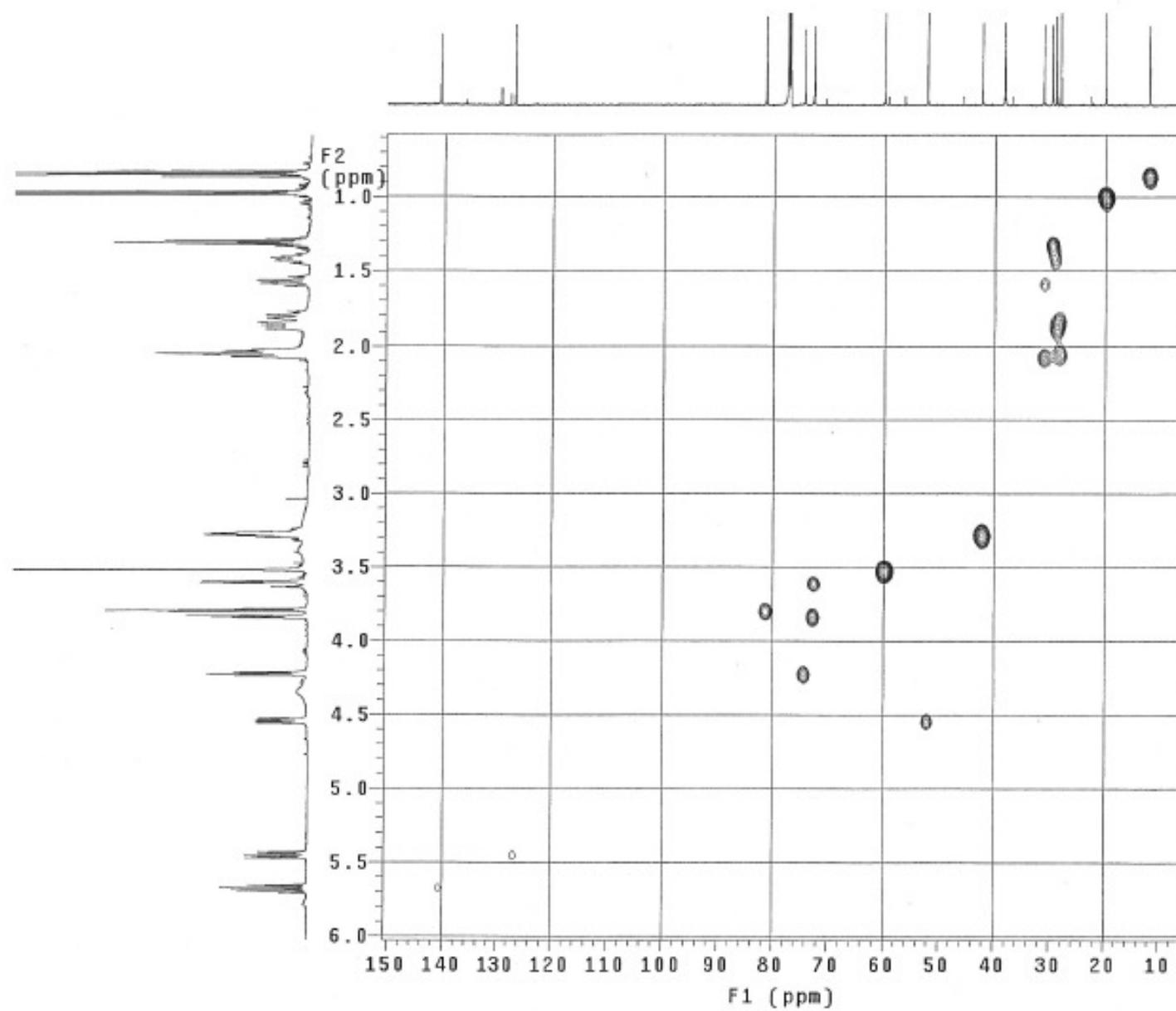
**Figure S7.** DEPT NMR spectrum of bengamide E' (**13**), (125 MHz, CDCl<sub>3</sub>) isolated from DSM 15898.



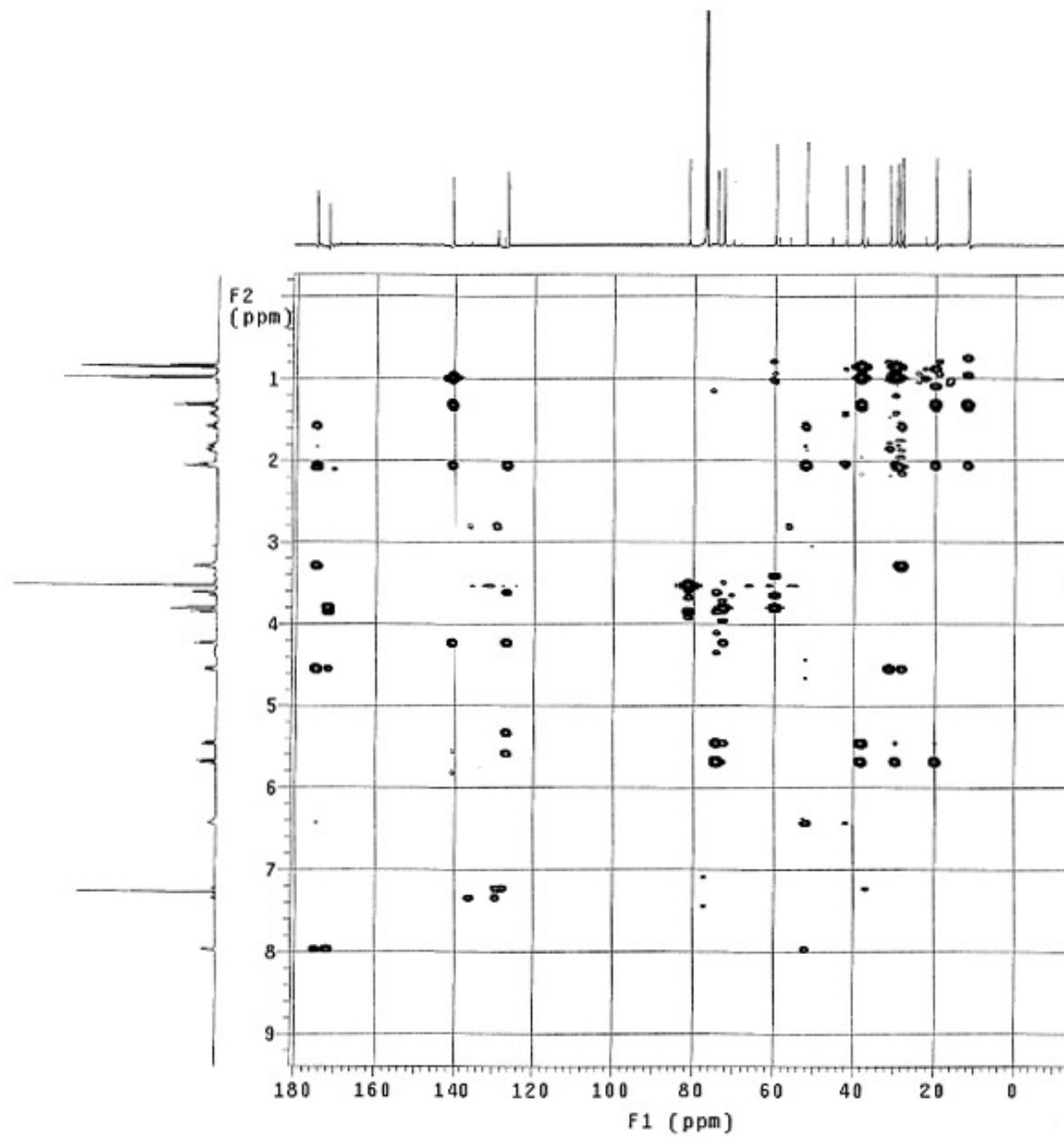
**Figure S8.** COSY NMR spectrum of bengamide E' (**13**), (600 MHz, CDCl<sub>3</sub>) isolated from DSM 15898.

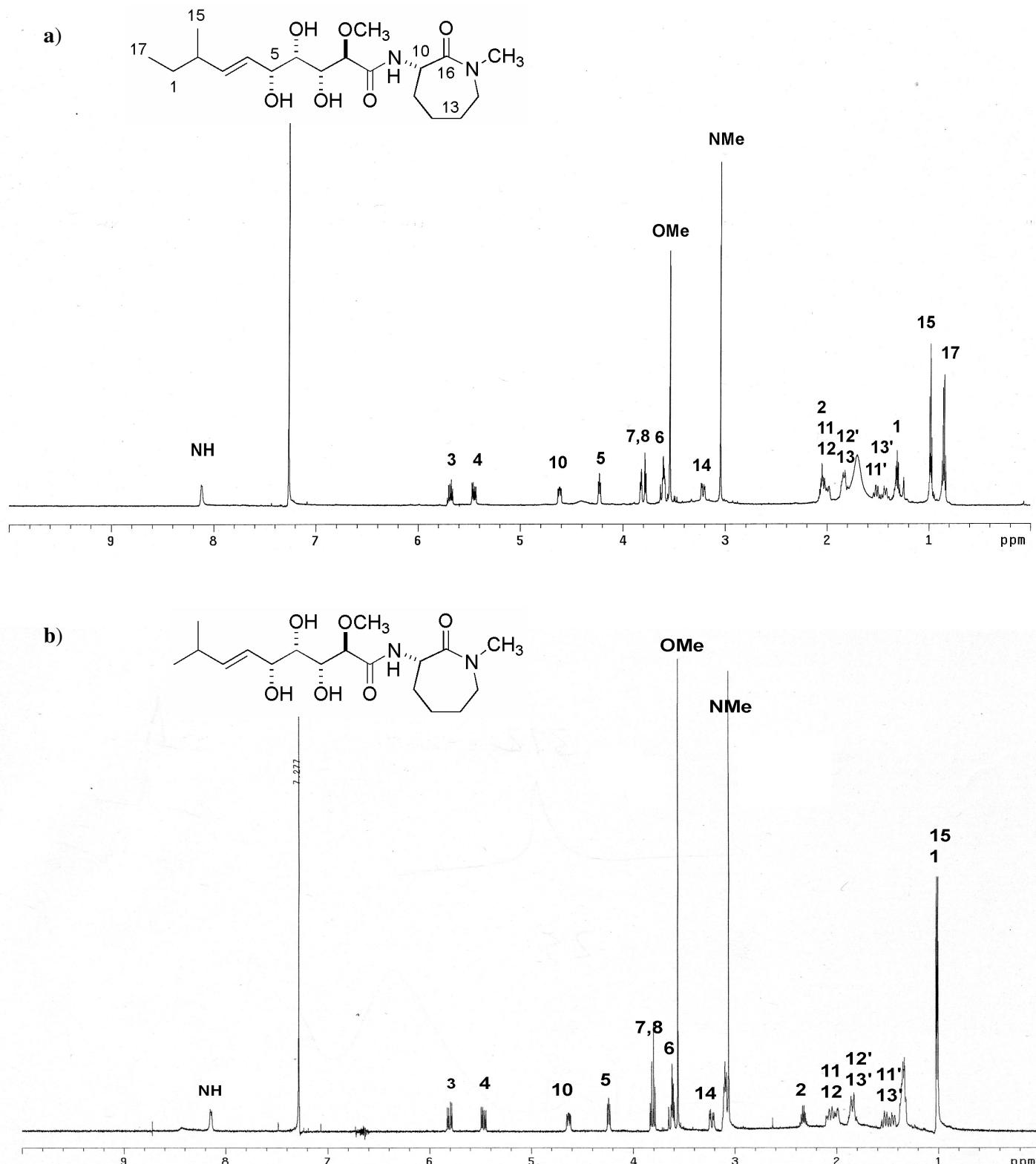


**Figure S9.** HMQC NMR spectrum of bengamide E' (**13**), (600 MHz, CDCl<sub>3</sub>) isolated from DSM 15898



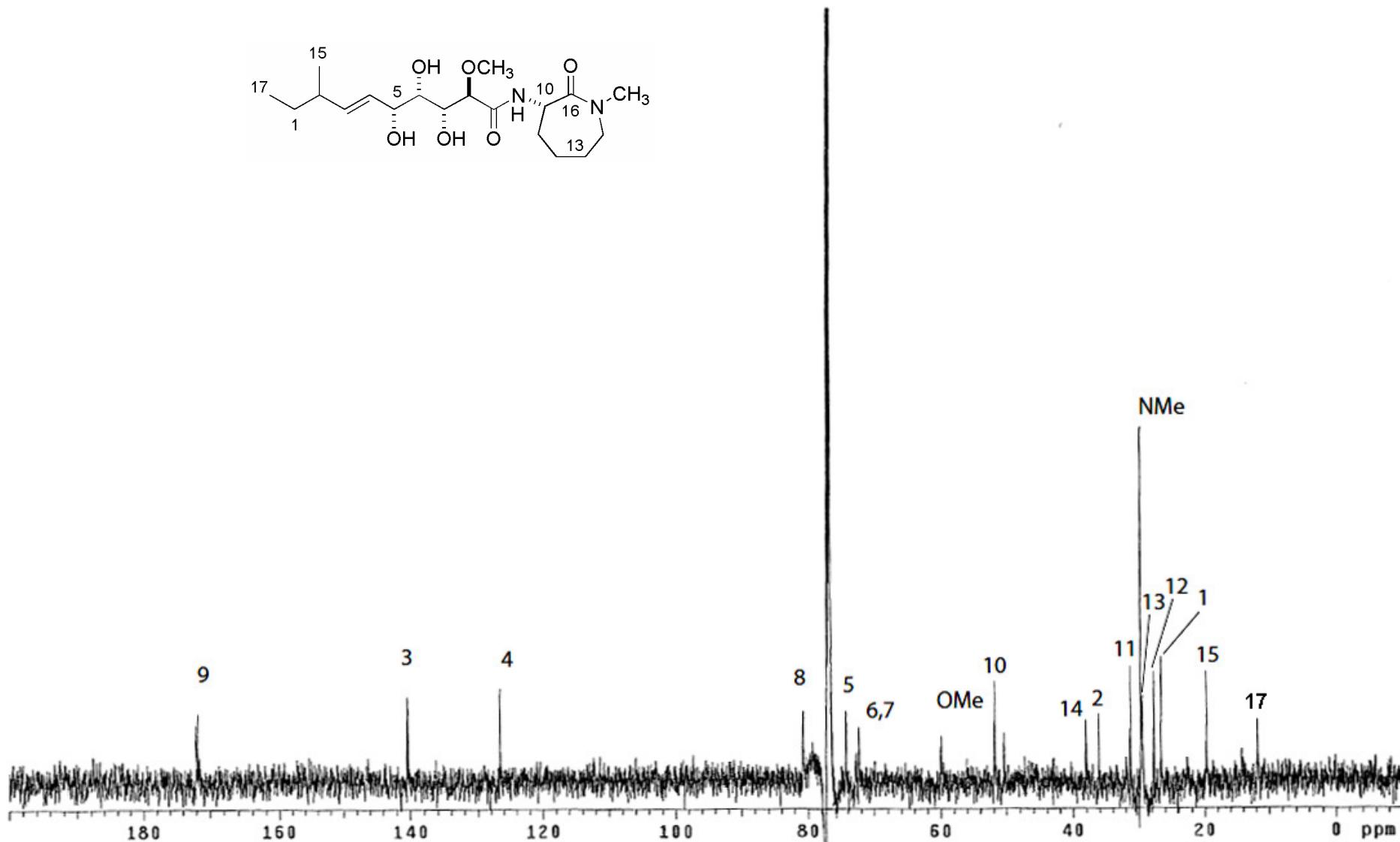
**Figure S10.** HMBC NMR spectrum of bengamide E' (**13**), (600 MHz, CDCl<sub>3</sub>) isolated from DSM 15898.



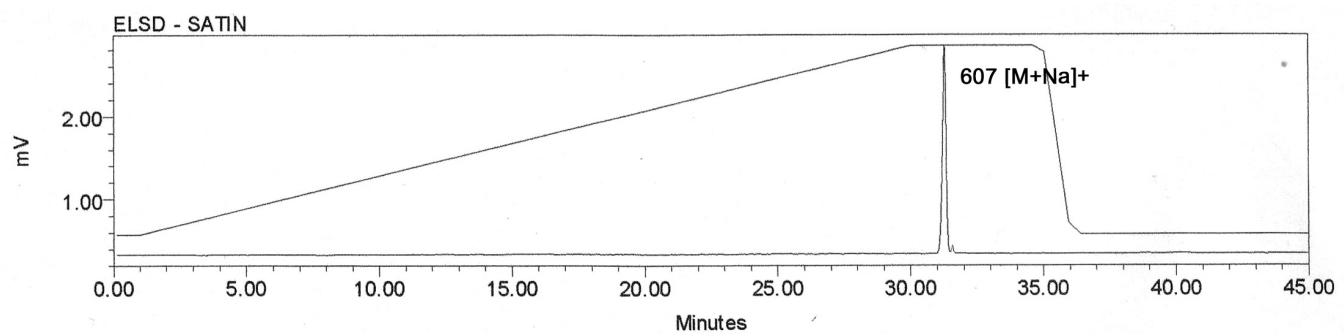


**Figure S11.** Comparative <sup>1</sup>H NMR spectrum of: (a) DSM 15898 FD P2H5H2 Bengamide F' (**14**) isolated from *M. virescens* versus (b) bengamide F (**9**) isolated from *J. coriacea* (600 MHz, CDCl<sub>3</sub>).

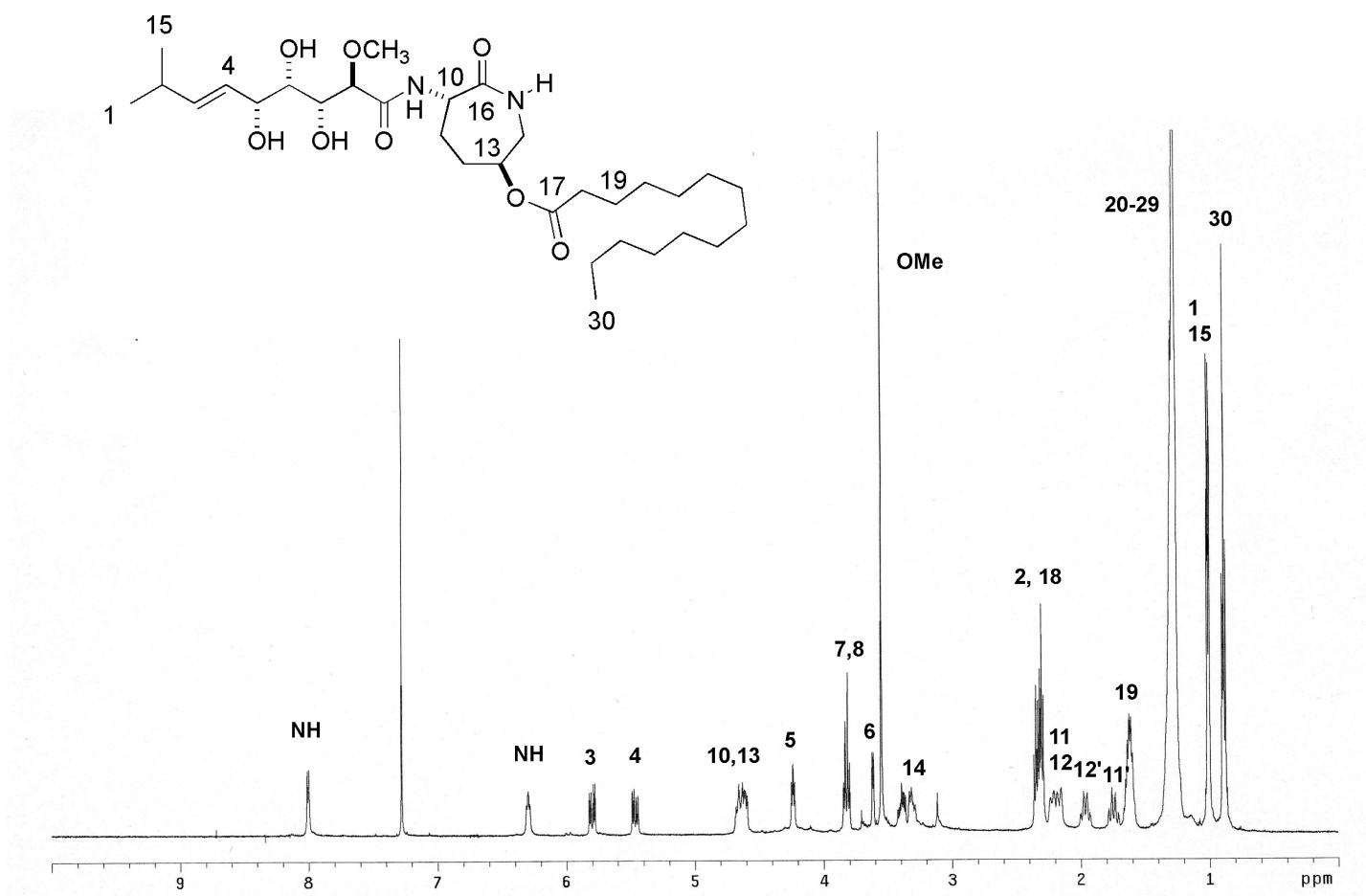
**Figure S12.**  $^{13}\text{CNMR}$  spectrum of bengamide F' (**14**), (125 MHz,  $\text{CDCl}_3$ ) isolated from DSM 15898.



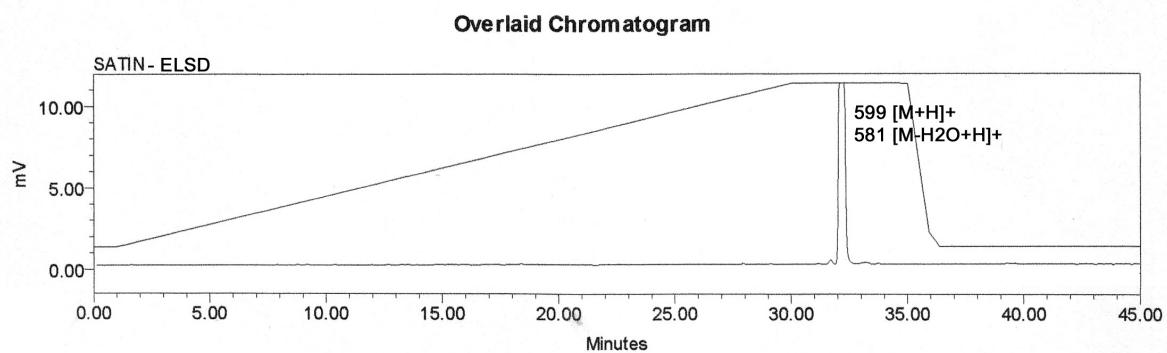
**Figure S13.** LCMS-ELSD trace of bengamide A (**6**) with annotations including  $m/z$  ions.



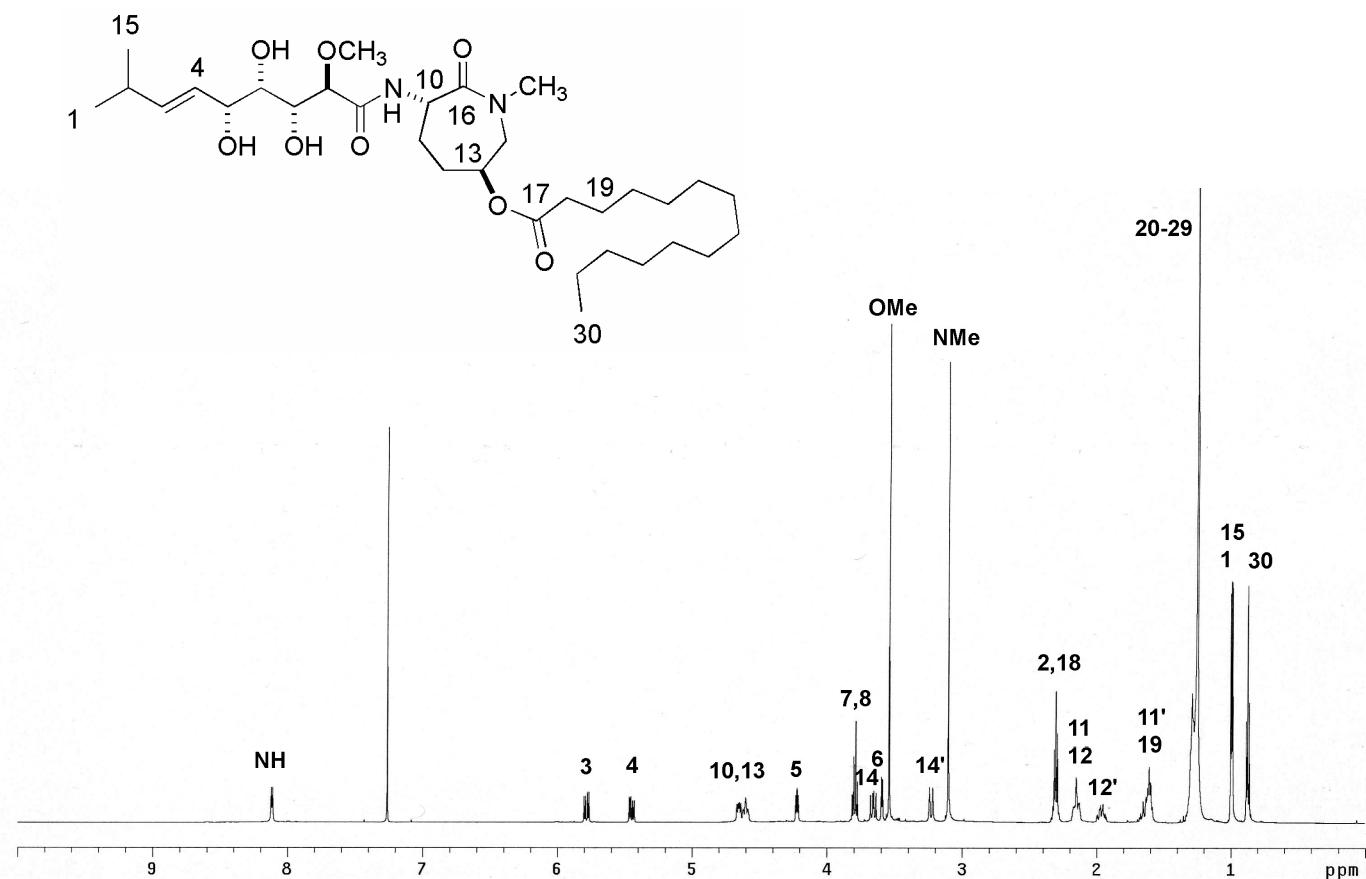
**Figure S14.**  $^1\text{H}$  NMR spectrum of bengamide A (**6**), (600 MHz,  $\text{CDCl}_3$ ) isolated from *J. coriacea*.



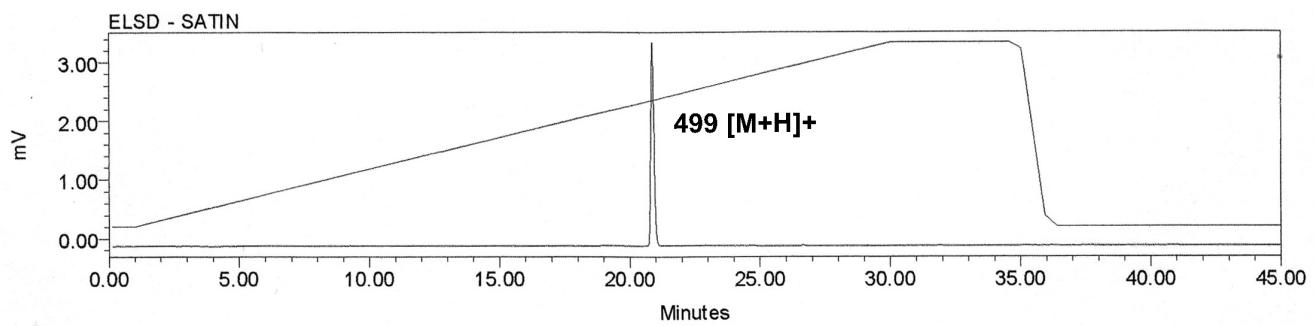
**S15.** LCMS-ELSD trace of bengamide B (**7**) with annotations including *m/z* ions.



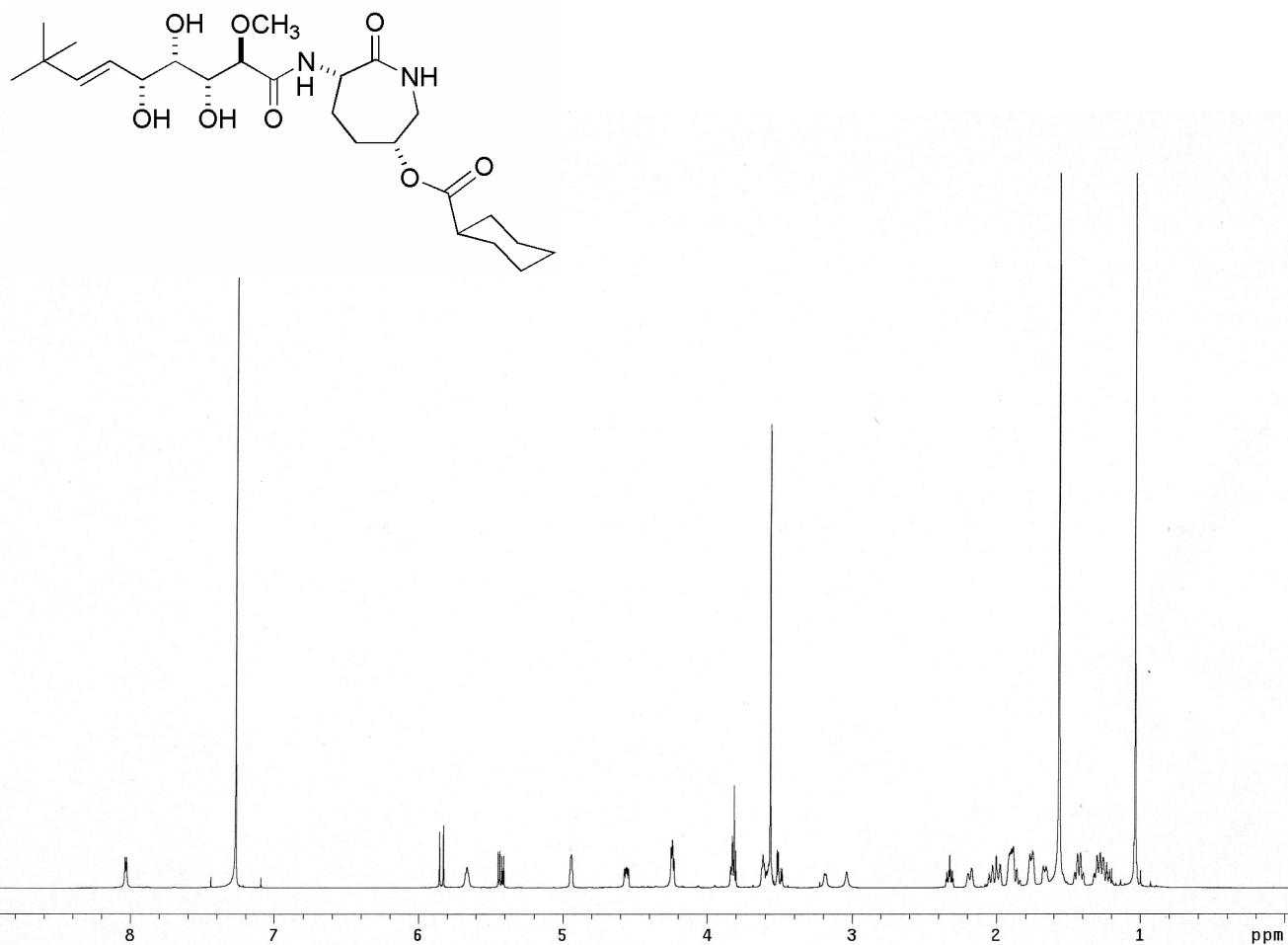
**Figure S16.**  $^1\text{H}$  NMR spectrum of bengamide B (**7**), (600 MHz,  $\text{CDCl}_3$ ) isolated from *J. coriacea*.



**Figure S17.** LCMS-ELSD trace of LAF 389 (**12**) with annotations including  $m/z$  ions.



**Figure S18.**  $^1\text{H}$  NMR spectrum of LAF 389 (**12**), (600 MHz,  $\text{CDCl}_3$ ).



**Figure S19.**  $^1\text{H}$  NMR analysis of diastereomers a and b of **13** (600 MHz,  $\text{CDCl}_3$ ,  $\text{C}_6\text{D}_6$ ).

